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## SHORT COMMUNICATION

### Evaluation of *Puccinia carduorum* for biological control of *Carduus pycnocephalus* in Tunisia

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*Puccinia carduorum*, a rust fungus from Italian thistle in Tunisia, was most aggressive on young growth stages of the weed in greenhouse tests. Repeated inoculations with the fungus significantly reduced weed biomass. Host-range tests suggest the fungus may be a safe biological control agent of Italian thistle in Tunisia.

**Keywords:** Italian thistle; rust fungus; classical biological control; Tunisia

*Carduus pycnocephalus* L. (Italian thistle) is a widely-distributed annual weed in northern Tunisia. It occurs in rangeland and pastureland as well as in crops. It spreads rapidly and is highly competitive. *Carduus pycnocephalus* can be controlled by mechanical cultivation or herbicides, however, these methods are not always effective or desirable because of weed seed longevity in the soil, inaccessibility of many sites to mechanical cultivation, development of resistance to some herbicides, and the undesirable impact of these chemicals on the environment (Schroder 1980). Alternatives such as biological control should therefore be considered.

Several investigators have reported the use of rust fungi as good candidates for classical biological control of weeds because of their host specificity and aggressiveness under favourable conditions. Successful biological control examples include: *Uromycladium tepperranum* on *Acacia saligna* (Morris 1997; Wood and Morris 2007); *Uromyces heliotropii* on *Heliotropium europaeum* (Delfosse, Lewis, and Hasan 1992); *Phragmidium violaceum* on *Rubus fruticosus* aggregate (Bruzzese and Hasan 1986; Evans, Jones, and Roush 2005); *Maravalia cryptostegiae* on *Cryptostegia grandiflora* (Tomley and Evans 2004) and *Puccinia chondrillina* on rush skeleton-weed, *Chondrilla juncea* (Hasan and Wapshere 1973; Emge 1981; Supkoff, Joley, and Marois 1988).

In 2008, the rust fungus *Puccinia carduorum* Jacky was first reported on *C. pycnocephalus* in Tunisia (Mejri, Souissi, and Berner 2008). Koch's postulates were fulfilled and an isolate was characterized molecularly (Mejri et al. 2008). This is an autoecious rust fungus which produces urediniospores and teliospores on many *Carduus* species (Baudoin, Abad, Kok, and Bruckart 1993). In field tests in the USA, the fungus accelerated senescence and reduced seed production of *C. nutans*

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by 20–57% (Baudoin et al. 1993) and was introduced into Virginia in 1987 for biological control of *C. nutans* (Baudoin and Bruckart 1996).

Our objectives were to: (1) determine the most susceptible growth stage of the plant to the fungus; (2) mimic secondary cycles of infection in nature, as simulated by repeated inoculations with urediniospores, and determine the effects of secondary cycles on weed biomass under greenhouse conditions; and (3) determine the susceptibility of two non-target species of economic importance in Tunisia. This would provide a better evaluation of the potential of this rust fungus as a biological control agent of *C. pycnocephalus*.

Eight plants in rosette stage, grown in pots containing a sterilized mixture of field soil, sand and peat (2:1:1, v/v/v), were inoculated with a suspension of urediniospores at  $10^6$  spores  $\text{mL}^{-1}$  and placed in a growth chamber at 18/20°C night and day temperatures. Urediniospores from diseased plants were used to inoculate 18 other plants at three different growth stages (2–5, 6–8 and more than 8 leaves) in order to determine the effect of plant age on the susceptibility of the weed. Disease incidence based on the proportion of plants diseased was recorded, and the evolution of the disease over time was estimated by the number of pustules on diseased leaves for each plant in each growth stage.

The effect of single and multiple inoculations of *P. carduorum* on *C. pycnocephalus* growth under greenhouse conditions was evaluated by inoculating rosettes one, two, three and four times, on a weekly basis, beginning 4 weeks after planting. Plants were inoculated with a suspension of urediniospores at  $10^6$  spores  $\text{mL}^{-1}$ ; controls received only water. Five plants per treatment were used. Dry weights of rosettes and roots were determined 3 weeks after the fourth inoculation.

The host range of *P. carduorum* tested in this study was restricted to two economically important related species: artichoke (*Cynara scolymus* L.) and safflower (*Carthamus tinctorius* L.). For each species, 8 plants were inoculated and 8 used as controls. The artichoke and safflower plants were inoculated at the 2-leaf stage and plants of *C. pycnocephalus* were inoculated at the 2–5-leaf stage. Plants were placed in a growth chamber at 18°C night and 20°C daytime temperatures, with a 16 h L:8 h D photoperiod until pustules developed. Pustule numbers per host plant were recorded over time.

Data were analyzed using Statistical Analysis System (SAS) software version 9.1, Cary, NC. Data were submitted to analysis of covariance to generate quadratic regressions of pustule numbers over time per plant. Means and standard errors of the mean were generated by analyses of variance for each day of observation. All experiments were repeated twice.

Out of the eight inoculated plants of *C. pycnocephalus*, six developed symptoms that were similar to those observed on samples collected during surveys (Mejri et al. 2008). Sixteen of the 18 plants inoculated at different growth stages developed symptoms and became diseased. The susceptibility of *C. pycnocephalus* to infection by *P. carduorum* significantly decreased with increasing plant growth stage (Figure 1). Sixteen days after inoculation, there were significantly more pustules per plant at the 2–5-leaf growth stage than the older ones (Figure 1).

Results of *P. carduorum* on *C. pycnocephalus* growth under greenhouse conditions showed significant reductions in root and rosette dry weights by an average of 33 and 42%, respectively, compared to controls. These reductions increased with multiple inoculations (Figure 2), and the development of pustules on diseased leaves increased

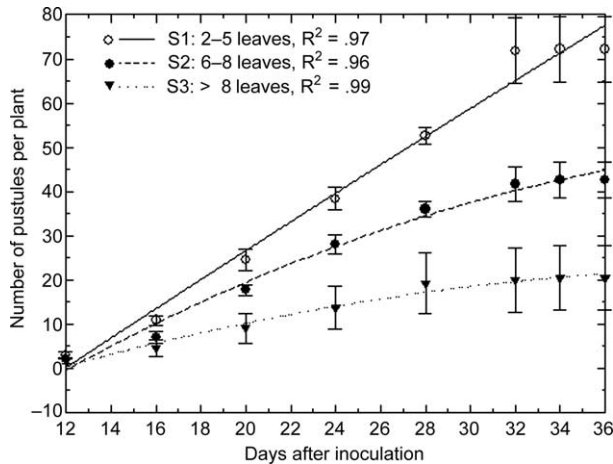


Figure 1. Mean values for the number of pustules developed on *Carduus pycnocephalus* plants inoculated at three plant growth stages. Standard errors of the means are indicated by vertical bars.

with the number of inoculations (data not shown). After four inoculations, root and rosette dry weights were reduced by 66 and 70% compared to controls. Based on this study, we suggest that secondary cycles of infection in nature, as simulated by repeated inoculations with urediniospores in this study, can reduce root biomass and therefore might exert a relatively high degree of control in nature (up to 70% reduction in rosette biomass based on our results).

Results of a limited host specificity test indicated that *P. carduorum* was unable to parasitize the two non-target related species. Only inoculated plants of *C. pycnocephalus* were severely diseased and disease incidence on the weed was 100%. No rust was observed on non-inoculated *C. pycnocephalus* plants or on artichoke and safflower plants. Pustules started developing on several leaves of inoculated plants of

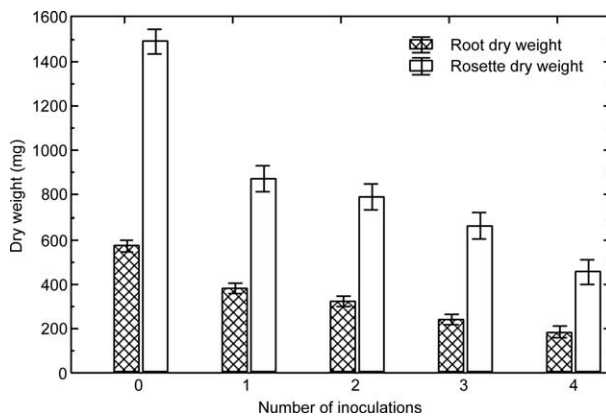


Figure 2. Average dry weight of roots and rosettes of *Carduus pycnocephalus* plants inoculated up to four times on a weekly basis with urediniospores of *Puccinia carduorum*. Standard errors of the means are indicated by vertical bars.

*C. pycnocephalus* 10 days after inoculation until they covered both the upper and lower surfaces of diseased leaves. One month after pustule appearance, diseased plants developed an average of 60 pustules on diseased leaves compared to no pustules on plants of the two related species.

Because *P. carduorum* is endemic to Tunisia, extensive host-range testing is not necessary to safely use the fungus in this country. Rather, means of augmenting naturally occurring inoculum and increasing distribution of the fungus to sites with *C. pycnocephalus* problems need to be developed. The simplest way of doing this may be through educating farmers about the rust and how and when to redistribute diseased plants (and consequently inoculum) into sites with problematic infestations of *C. pycnocephalus*.

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